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MINIREVIEW

The Laboratory Diagnosis of Syphilis

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ABSTRACT Syphilis is a multisystem infection caused by the spirochete *Treponema pallidum*. Currently, cases of possible syphilis are commonly investigated using the treponemal serological tests *T. pallidum* IgG chemiluminescence immunoassay (CLIA) and the *T. pallidum* particle agglutination (TPPA). The nontreponemal rapid plasma reagin (RPR) flocculation test is used to assess disease activity. There has been a resurgence of syphilis diagnoses in Australia. Large foci of infection have been identified in isolated communities. The remoteness of these locations, in conjunction with the particular sociocultural characteristics of the population, pose unique challenges to the traditional diagnostic and treatment paradigms for syphilis. As a consequence of this increased incidence of syphilis, there has been interest in the utility of point-of-care tests (POCTs), nucleic acid amplification tests (NAATs), the role of IgM testing in suspected congenital syphilis, and the laboratory investigation of possible neurosyphilis. This review looks at the current status of traditional serological assays and provides an update on more recent methods. It assesses the published literature in this area and makes recommendations for the rational use of pathology testing to aid in the diagnosis of the many facets of syphilis.

KEYWORDS diagnostics, syphilis

Syphilis is a multisystem infection caused by *Treponema pallidum* subsp. *pallidum*. It is commonly sexually transmitted but can also be vertically transmitted during pregnancy, causing congenital syphilis (1–4). Globally, there are approximately six million new cases of syphilis annually in persons aged between 15 and 49 years. Internationally, >300,000 fetal and neonatal deaths are attributed to syphilis, with an additional 215,000 infants at increased risk of early death (5).

The natural progression of untreated syphilis is divided into primary, secondary, and latent stages (2–4, 6, 7). Primary syphilis typically presents as a spontaneously resolving painless ulcer (chancre) at the site of inoculation. It is followed by systemic dissemination of *T. pallidum*, manifesting frequently as a widespread maculopapular rash and nonspecific systemic symptoms that characterize the secondary stage, which resolves without treatment. The latent stage is divided into early (<2 years duration) and late (>2 years) and refers to the state where the disease does not manifest any symptoms. When it becomes symptomatic (tertiary), it can involve virtually any organ, including the central nervous system (neurosyphilis), skin, bone (gumma), and the cardiovascular system. Neurosyphilis, however, can manifest during any stage of the disease (3, 7).

The laboratory diagnosis of syphilis, especially in congenital syphilis and neurosyphilis, continues to pose challenges. The interpretation of each test varies and depends upon the stage of the disease. Historically, serology has been the main diagnostic method but continues to have issues with the lack of specificity of nontreponemal tests and a poor correlation of treponemal tests with disease activity. Direct detection

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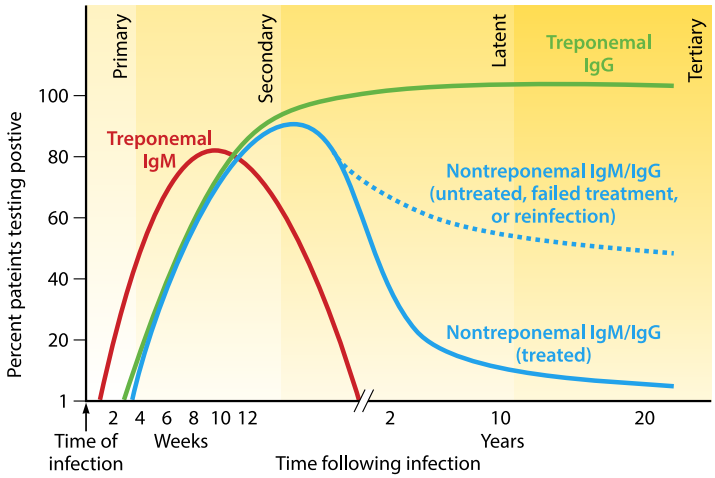

FIG 1 Progression of syphilis serology throughout the stages of the disease (reproduced from reference 6 with permission of Elsevier).

methods such as molecular or specialized microscopy techniques are also limited by their performance, availability, and cost.

This review aims to provide a comprehensive summary on the diagnostic tests available for syphilis along with their performance throughout the stages of the disease, including neurosyphilis, and in congenital syphilis.

SEROLOGICAL TESTING FOR SYPHILIS

Serological tests for syphilis consist of treponemal and nontreponemal tests. These have been the main method for screening, diagnosis, and monitoring of disease activity. Figure 1 highlights the progression of treponemal and nontreponemal antibodies throughout the disease (6). A summary of the performance of these assays in each stage of the disease is provided in Table 1.

Treponemal tests. Treponemal tests are qualitative assays performed on serum to detect antibodies (usually IgG but occasionally IgM) against a variety of *T. pallidum* antigens; these antibodies are detectable 2 to 4 weeks after exposure (6, 8). Results are typically reported as reactive or nonreactive without any titers. Treponemal tests are, in general, more sensitive in early infection, and once positive, they usually remain reactive indefinitely (3). They cannot be used to monitor treatment response or diagnose reinfection due to poor correlation with disease activity (4, 8, 9). They also cannot distinguish syphilis from infections with endemic *Treponema*, such as yaws (*T. pallidum* subsp. *pertenue*) and pinta (*T. carateum*) due to treponemal species cross-reaction (8, 10, 11).

(i) Enzyme immunoassay and chemiluminescence immunoassay IgG/IgM. The enzyme immunoassay (EIA) is an antitreponemal assay commonly used as an automated screening test. Many commercial assays use recombinant treponemal antigens (Tp15, Tp17, and Tp47) to detect IgM, IgG, or both (3). It is available in both sandwich capture and competitive assay formats. The overall sensitivity and specificity are comparable to those of the *T. pallidum* particle agglutination assay (TPPA) or fluorescent antibody-absorption (FTA-ABS) (10), although a high background signal can give false-positive results (2). The chemiluminescence immunoassay (CLIA) is a variation of the EIA. It is a rapid, high-throughput automated assay that utilizes paramagnetic particles coated with recombinant antigen to capture IgM and/or IgG followed by addition of a chemiluminescence substrate to generate a signal relative in proportion to the amount of the bound antigen-antibody complex (2). The turnaround time is less than 1 h (2). The availability of automation in both the EIA and CLIA makes them the mainstay of syphilis screening in a busy diagnostic laboratory.

TABLE 1 Summary of syphilis testing performance

Test[a]	Sensitivity (% [range]) for disease stage				Specificity (% [range])	Reference(s)
	Primary	Secondary	Early latent	Late latent		
Serology (nontreponemal)						
VDRL	78 (74–87)	100	96 (88–100)	71 (34–94)	98 (96–99)	37
RPR	86 (77–99)	100	98 (95–100)	73	98	37
USR	80 (72–88)	100	95 (88–100)	NA	99	37
TRUST	85 (77–86)	100	98 (95–100)	NA	99 (98–99)	37
Serology (treponemal)						
FTA-ABS	84 (70–100)	100	100	96	96 (95–100)	37
TPPA	88 (86–100)	100	100	94	96 (95–100)	10
TPHA	86	100	100	99	96	38
MHA-TP	76 (69–90)	100	97 (97–100)	94	99 (98–100)	37
EIA						
EIA IgG	100	100	100	NA	98	39
EIA IgM	93	85	64	NA	95	40
CLIA	98	100	100	100	99	41
Direct detection (skin, mucosa, and exudates)						
Dark-field microscopy	84 (71–100)	60 (25–100)			92 (88–100)	24, 42, 43
Silver stain histochemistry	86 (50–100)	40 (0–92)		4 (0–11)		42
Immunohistochemistry	100	87 (58–100)		36 (11–60)	100	42
PCR (tissue)	100	67 (42–100)		7 (0–14)		42
PCR (lesional smear)	90 (80–96)	83 (80–86)			98 (96–100)	24, 42

[a]VDRL, Venereal Disease Research Laboratory; RPR, rapid plasmin reagin; USR, unheated serum reagin; TRUST, toluidine red unheated serum test; FTA-ABS, fluorescent antibody absorption; TPPA, *T. pallidum* particle agglutination; TPHA, *T. pallidum* hemagglutination; MHA-TP, microhemagglutination assay for *T. pallidum*; EIA, enzyme immunoassay; CLIA, chemiluminescence immunoassay.

(ii) Immunoblot assay. This is used primarily as an adjunct confirmatory test to resolve any inconclusive results from other treponemal tests. It is a Western blot, highly specific and able to detect IgM and IgG separately (3, 10). It is an original immunoblot that utilizes the whole-cell organism as the antigen and detects antibodies to the major surface antigens of *T. pallidum* (TpN15, TpN17, TpN44.5, and TpN47). It is laborious and can be difficult to interpret due to nonspecific reactions (3, 12). It has been superseded by recombinant immunoblot assays that are available commercially, such as the INNO-LIA Syphilis (Innogenetics NV, Ghent, Belgium), ViraBlot (Viramed Biotech, Planegg, Germany), and MarDx test (Trinity Biotech, Bary, Ireland).

The INNO-LIA Syphilis kit is a line immunoassay that detects three recombinant antigens (TpN15, TpN17, and TpN47) and one synthetic peptide with sensitivity and specificity approaching 100% (3, 12). In addition to these antigens, control lines are used to assess performance of the reagents and for semiquantitative evaluation of the results (3). ViraBlot uses similar recombinant antigens as INNO-LIA and an additional Venereal Disease Research Laboratory (VDRL)-specific antigen (2). Both INNO-LIA and ViraBlot have better performance and concordance with other treponemal assays (EIA, FTA-ABS, and TPPA) than the MarDx test, which uses whole *T. pallidum* lysate (2); INNO-LIA requires overnight incubation, while ViraBlot and MarDx can be completed within 2 h (13).

(iii) Fluorescent antibody-absorption IgG/M. This is an indirect immunofluorescent assay which involves pretreatment of serum with an absorbent, usually an extract of the nonpathogenic *Treponema phagedenis*. This is to remove potential nonspecific cross-reactive antibodies from test sera. Dilutions of test sera are then added to slides dotted with fixed organisms from an extract of *T. pallidum* (Nichols strain) culture. Fluorescent-conjugated anti-human immunoglobulin is then used to visualize antibody-labeled organisms (2, 9, 10). The test usually takes 1.5 h to complete. The FTA-ABS is not recommended as a routine screening test for syphilis due to its subjective interpretation, inability to automate for large specimen numbers, and the requirement for a fluorescence microscope. Nonspecific reactions can still occur, giving a false-positive result (2, 9).

(iv) Microhemagglutination assay, *T. pallidum* hemagglutination assay, and *T. pallidum* particle agglutination assay. The microhemagglutination assay for *T. pallidum* (MHA-TP) and *T. pallidum* hemagglutination assay (TPHA) are manual indirect hemagglutination assays performed in microtiter plates using sheep and fowl erythrocytes sensitized with *T. pallidum* antigen, respectively, which agglutinate with anti-treponemal IgM and IgG antibodies (2, 10). Serum is first mixed with absorbing diluent made from nonpathogenic Reiter treponemes and other absorbents to reduce potential false-positive reactions (12). Serum containing the antibodies will cross-link the red blood cells and form a smooth mat on the base of the well (12).

The TPPA has replaced both the MHA-TP and TPHA, as it has better sensitivity and comparable sensitivity to FTA-ABS (2, 9, 10). It is also performed in microtiter plates and typically requires a minimum 2 h of incubation (2). It is easier to perform and less prone to nonspecific reaction by using sensitized gelatin particles instead of erythrocytes (2, 9). Unsensitized gelatin particles are used as a control for nonspecific agglutination. Compared to that of the FTA-ABS, it has comparable sensitivity but is less expensive and complicated, with the results interpretable to the naked eye (9).

(v) Multiplex flow immunoassay. This is an automated flow cytometric assay based on Luminex fluorescent bead technology that enables high throughput. It is similar in principle to the EIA, but the capture antibody is attached to polystyrene beads instead of a well. Using dual laser flow cytometry, the identity and amount of capture antibody are determined by the fluorescence signature of the dyed beads and their intensity (2).

Two commercial assays are available: BioPlex 2200 Syphilis (Bio-Rad Laboratories, Hercules, CA) and the AtheNA Multi-Lyte assay (Zeus Scientific, Branchburg, NJ). The BioPlex 2200 is capable of simultaneous detection and differentiation of treponemal antibodies (IgG and IgM) against recombinant Tp15, Tp17, and Tp47 antigen and nontreponemal antibodies (rapid plasma reagin [RPR]) along with its titer (2). The AtheNA Multi-Lyte assay detects IgG only against recombinant Tp17 (2).

(vi) Anti-treponemal IgM. Anti-treponemal IgM can be an indicator of recent infection in adults and in neonates. After treatment, it usually declines at around 4 months but may remain elevated longer if the disease is untreated (6, 7). The detection of anti-treponemal IgM is largely confined to selected cases, mainly for the diagnosis of congenital syphilis. The methods for anti-treponemal IgM detection include the FTA-ABS IgM, immunoblot, and EIA IgM (9). Commercial EIAs are available. A reactive anti-treponemal IgM may indicate active syphilis but does not preclude the possibility of chronic infection due to prolonged release of IgM (6). Furthermore, a nonreactive result does not exclude recent infection. The use of the anti-treponemal IgM in the diagnosis of congenital syphilis is discussed in a later section of this review.

Nontreponemal tests. Nontreponemal tests are performed on serially diluted serum to detect total antibodies (IgM and IgG) directed against lipoidal antigens, such as cardiolipin and lecithin, which are released from damaged host cells and the bacteria (2, 4, 6, 9). These antibodies are nonspecific and usually not detectable until a few weeks after infection (Fig. 1) (6). Examples of nontreponemal tests include the Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), unheated serum reagin (USR), and toluidine red unheated serum test (TRUST) methods (2, 4). As opposed to treponemal tests, nontreponemal tests are quantitative and reported in titers.

The VDRL is a microflocculation test to detect antibodies in patients' sera against reagin, i.e., an antigen formed by a combination of cardiolipin, lecithin, and cholesterol. It requires microscopy ($\times$100 magnification) for interpretation (9). It is, to date, the only acceptable nontreponemal assay that can be performed on cerebrospinal fluid (CSF) for the diagnosis of neurosyphilis (2, 9, 10). It can be subjective to interpret and requires daily preparation of the antigen suspension and preheated serum at 56°C for 30 min to inactivate complement (10, 14). Preheating is not required for CSF. The USR is a modified VDRL that uses stabilized antigen by adding choline chloride and EDTA to serum, and so heat pretreatment of serum is unnecessary (9, 10, 15).

The RPR is a macroflocculation card-based test that uses the same antigen as VDRL except that it is bound to a carbon particle and uses charcoal particles as the visualizing agent. A positive reaction appears as a black clump against a white background. The RPR is one of the most commonly performed nontreponemal tests (2, 9). The TRUST is similar to the RPR but uses toluidine red as a visualizing agent instead of charcoal. It has similar sensitivity but slightly better specificity (2).

Nontreponemal tests are used to monitor disease activity. A 4-fold or greater decrease in the follow-up titer from the baseline titer tested in parallel using the same assay, for example, from 1:8 to 1:2, or seroreversion indicates successful treatment (11). Occasionally, a serofast state (persistent low-positive nontreponemal antibody) can occur despite appropriate treatment (11). This is seen in two groups: patients who achieved serological cure with a 4-fold decline in their titers and patients in whom titers do not achieve a 4-fold decline, i.e., serological nonresponders (16). The cause of serological nonresponse is unclear and may not be driven by active syphilis. The clinical significance is also unknown (16). Several studies have shown that patients with serological nonresponse do not have worse clinical outcomes in the short term. Additional antibiotic therapy does not appear to improve the serological responses or alter clinical outcomes, although the long-term implications remain undefined (16).

Similarly, the titer from nontreponemal tests is useful for the diagnosis of reinfection or relapse, as highlighted by 4-fold or greater increase in titer (7). Moreover, without treatment, the titer from nontreponemal tests generally remains positive at low titers or may revert to negative (4). The titers from nontreponemal tests are not interchangeable between methods used. Testing should therefore ideally be performed in parallel using the same method in the same laboratory.

Nontreponemal tests are not automated and rely on subjective interpretation. Biological false positives (BFPs) due to cross-reactivity can occur in association with many conditions. These include but are not limited to a variety of infectious and noninfectious diseases such as connective tissue and autoimmune disease, ulcerative colitis, vasculitis, chickenpox, hepatitis, infectious mononucleosis, measles, mumps, viral pneumonia, bacterial endocarditis, brucellosis, malignancy, and intravenous drug use (3, 10). Pregnancy has also been cited as a potential cause of a BFP nontreponemal test (3, 10). False negatives may also occur with a prozone phenomenon, whereby a high antibody titer interferes with antigen-antibody complex formation. It can be resolved by diluting samples to at least a 1:16 dilution (2, 9).

Diagnostic algorithms for the serological diagnosis of syphilis. There are two main syphilis testing algorithms: the "traditional" and "reverse" algorithms (6, 11). The traditional algorithm uses the nontreponemal assay for primary evaluation followed by a confirmatory treponemal assay for reactive samples only. As nontreponemal assays are less sensitive in early and latent disease, there is a potential risk of underdiagnosis (9, 17). Therefore, a treponemal test should be performed when suspecting these stages of infection despite a nonreactive nontreponemal test. The other limitations of this algorithm include the lack of specificity, manual operation, and subjective interpretation of the result (10). It is, however, more cost effective and, hence, suited to resource-limited settings and low-volume laboratories (9, 17).

Conversely, the reverse algorithm uses a treponemal assay first followed by a nontreponemal assay for reactive samples. When a new diagnosis of syphilis is made or if a discordant result is obtained, (i.e., positive treponemal with negative nontreponemal test), a second treponemal assay using a different platform is performed for confirmation (9, 17). The TPPA is generally the recommended confirmatory test on discordant samples (6, 9, 11). The automated EIA, CLIA, and multiplex flow immunoassay (MFIA) are commonly used as screening tests in high-volume laboratories, as they are less labor intensive and more efficient, with rapid turnaround times (4, 8). They allow for detection in early disease but have a limitation with an increased risk of false positives in a low-prevalence population (8, 10). The use of a second confirmatory treponemal test is performed to reduce the false-positive rate (9).

Multiple factors, including local syphilis prevalence, volume of testing, cost, and workload, are essential before implementation of the algorithms (17).

Interpretation. The serological diagnosis and exclusion of syphilis, in most suspected cases with high clinical probability, require collective results from both treponemal and nontreponemal tests (10). As mentioned, the sensitivities of both tests are dependent on the stages of the disease. Treponemal tests are more sensitive in early disease (3). In most instances, they remain detectable for prolonged periods, although seroreversion on TPPA has been reported in several studies, with rates of 11% to 13%, and was associated with HIV coinfection (18, 19). Nontreponemal tests are less sensitive in early and latent disease and more prone to BFPs (2, 9, 10). The clinical context should always be considered in high-risk groups with a high pretest probability of the disease. A negative or discordant serology should prompt repeat testing. False-negative treponemal tests can occasionally occur in patients with immunosuppression, including those with HIV. However, in general, the serologic tests perform well and are reliable in these cohorts (2).

Syphilis point-of-care tests. Most syphilis point-of-care tests (POCTs) use an immunochromatographic method that utilizes impregnated recombinant *T. pallidum* antigen on a test strip that produces a visible line from an antigen-antibody reaction and provides results within 30 min (2, 9). Appropriate specimens for POCTs are serum, plasma, and whole blood (10). Compared with the TPPA, the sensitivity and specificity of most POCTs are 85% to 98% and 93 to 98%, respectively (1). There are many commercially available POCTs, but only one treponemal POCT (Syphilis Health Check; Trinity Biotech, Jamestown, NY, USA) is currently US Food and Drug Administration (FDA) approved (20). As with other anti-treponemal antibody tests, a positive treponemal POCT does not distinguish between active or past infection (1, 4, 9). Consequently, the treatment of all positive results could result in unnecessary treatment (1, 9). Nevertheless, given the significant outcomes of untreated disease, the benefits of overtreatment likely outweigh any potential harm, especially in high-risk groups (1).

Dual treponemal/nontreponemal POCTs were designed to address this problem. The DPP Syphilis Screen and Confirm Assay (Chembio Diagnostic System Inc., Medford, NY, USA) is a commercially available immunochromatographic assay with this feature (3, 4, 9, 21). Both the treponemal and nontreponemal results are qualitatively reported as either reactive or nonreactive on separate lines. The sensitivity of the nontreponemal test is dependent on the level of the RPR titer. An RPR titer of $\geq$1:2 results in better concordance with the dual POCT up to 98%. This decreases to 88% to 90% when the RPR is $\leq$1:1 (21). The sensitivity of treponemal POCTs compared to that of standard laboratory tests varied between studies from 85% to 98%, and the specificity was found to be between 93% and 98% (1). Commercial dual syphilis/HIV POCTs that can detect both diseases using one single specimen are also available and FDA approved (2, 4, 22).

The POCT is generally simple to perform, requiring only a small amount of blood from a finger prick, and is easy to interpret (visual readout in a card or strip format). It is cost effective with minimal requirement for storage, transport, equipment, or training (2, 5, 10). The main advantage is the rapid result allowing prompt treatment with a subsequent reduction in untreated disease and transmission (5, 8, 9, 11). This is particularly beneficial in a cohort of patients at high risk of infection who are unlikely to attend follow-up or in low-resource setting where laboratory capacity or access is limited (1). Immunochromatographic POCT has also been shown to be cost effective for screening of maternal syphilis in low-resource settings compared to screening with a traditional algorithm or nontreponemal test alone (9).

The performance of the syphilis POCT has been reported to be comparable to that of standard syphilis serological testing, although it might potentially be lower when used in the field (8, 15, 21). There is also limited data on their performance in HIV-infected individuals or in those with high RPR titers, with a theoretical concern for prozone effect (9). Despite its simplicity, the result of a POCT can be subject to errors due to misinterpretation, an inexperienced operator, and variability in the test lots. Lack of

quality assurance and governance can be major issues. Therefore, a written policy/procedure should be developed in conjunction with the local laboratory, which can oversee ongoing quality assurance and troubleshoot problems (7, 9, 15). Staff education and training remain essential.

DIRECT DETECTION METHODS

Culture. *T. pallidum* cannot be cultured on routine laboratory culture media (2, 10). The rabbit infectivity test, which involves inoculating a clinical specimen into the testes of live rabbits, is the only available method for isolation of *T. pallidum*. It is impractical for routine diagnosis due to the time and cost, the need for trained personnel, and the ethical issues of using live animals (2). Some *T. pallidum* strains can be propagated continuously *in vitro*, but this has not yet been done in clinical samples.

Nucleic acid amplification testing. As *T. pallidum* cannot be grown on routine culture, molecular assays with nucleic acid amplification tests (NAATs) of *T. pallidum* DNA are used for direct detection to improve diagnostic sensitivities. Methods of NAATs include the PCR, nested PCR, quantitative PCR, and reverse transcriptase PCR (23). The most frequently used and evaluated target genes are the *T. pallidum* 47-kDa lipoprotein (*tpp47*) and the DNA polymerase I gene (*T. pallidum* polymerase A gene [*polA*]) (2, 23, 24). Other target genes are treponemal membrane protein A (*tmpA*), subsurface lipoprotein 4D (*4D*), and basic membrane protein (*bmp*) (2, 23, 24). None of these assays are as yet commercially available (4). This limits their use to most laboratories that have validated in-house assays (2, 10, 23).

T. pallidum PCR is complementary to serology in the diagnosis of early syphilis, reinfection, and congenital syphilis (12). The specificity ranges from 97% to 100%, but the sensitivity varies depending on specimen type and stages of infection (12). The highest overall sensitivity of NAAT detection for *T. pallidum* was seen in primary lesions ranging from 75% to 95% with the *tpp47* target and 72% to 87% with the *polA* target but reduced in lesions from secondary syphilis, such as mucous membrane or maculopapular lesions and condyloma lata, to 20% to 86% with the *tpp47* target and 43% with the *polA* target (23). They are, however, insensitive for detection from whole blood or blood fractions and are therefore not recommended (23).

A comparison of the utility of PCR of genital lesions with syphilis serology, however, showed that up to 97% of patients with a lesion that was PCR positive for *T. pallidum* were simultaneously seropositive, which would suggest a limited role for the more costly PCR detection over serology in the diagnosis of this condition (25).

The use of NAATs in the diagnosis of neurosyphilis is discussed in a later section.

Dark-field microscopy. Wet mounts of exudates from lesions are examined using dark-field microscopy (DFM) to identify the characteristic morphology and motility of live *T. pallidum* (11). It is particularly useful in early syphilis, when antibodies are not yet detectable. Although it can potentially be a useful POCT within a sexually transmitted disease clinic, it requires a dark-field microscope and trained personnel in proximity to patients, which is difficult to set up (8, 11, 23). Time is also essential before motility disappears, requiring the sample to be examined within 20 min (23). Apart from moist syphilitic lesions, DFM is usually not performed on other body sites due to a low treponemal burden. It should not be performed on oral lesions, as it is difficult to distinguish *T. pallidum* from oral spirochetes (8). A negative finding does not exclude the diagnosis (10). For these reasons, DFM is rarely performed outside reference laboratories.

Direct fluorescent antibody for *T. pallidum*. Direct fluorescent antibody for *T. pallidum* (DFA-TP) is an immunofluorescence assay that utilizes monoclonal or polyclonal antibodies to detect *T. pallidum* antigens directly from the specimen (11, 12). The specimen is collected as per DFM but requires fixation to microscopy slides followed by incubation with the conjugated antibody. Body fluids may need centrifugation to concentrate the bacteria before fixation (11). Immunofluorescent microscopy is required for interpretation (11, 12). As opposed to DFM, the DFA-TP is not dependent on motility and is easier to interpret. It is also able to distinguish between *T. pallidum* and nonpathogenic spirochetes but not other pathogenic treponemes (10). Despite the

availability of commercial monoclonal or polyclonal antibodies, they have not been validated for clinical use and are not FDA cleared. This method is also limited by its technical complexities and instrument requirement (11).

Histology and immunohistopathologic staining. Syphilis causes histologic features of the affected tissue depending on the type of lesion and the stage of the disease. Primary syphilis is usually characterized by a chancre, the histology of which shows an acanthotic epidermis that becomes ulcerated with time, dense lymphocytic and plasma cell infiltration, and endothelial swelling (26, 27). Spirochetes are usually identified within or around blood vessels and at the dermal-epidermal junction. There is significant variability in the histologic features of secondary syphilis. The epidermis can be normal, necrotic, or ulcerated, while the dermis can show changes resembling primary syphilis with a dense infiltrate of lymphocytes and endothelial swelling. A lichenoid tissue reaction can also be present (26, 27). Tertiary syphilis can be characterized by local inflammation or development of a necrotizing granulomatous lesion (gumma) in the affected tissues, such as skin, bone, and aorta (26, 27).

Silver and histological stains can be utilized to highlight the spirochetes in formalin-fixed paraffin-embedded tissue from lesions of primary and secondary syphilis (2, 23). They are not useful in tertiary disease. Silver staining, such as the Steiner or Warthin-Starry stain, has limited sensitivity and specificity, as melanin and reticulin fibers can be misinterpreted as spirochetes (8). Compared to the silver stain, an immunohistochemistry stain offers better sensitivity and specificity (26).

CONGENITAL SYPHILIS

Congenital syphilis occurs following transplacental transmission of *T. pallidum in utero*. Both primary and secondary syphilis confers a highest risk of transmission (60% to 100%). The estimated rates of vertical transmission for early latent and late latent are 40% to 83% and 10%, respectively (3). The consequences are variable. These include asymptomatic infection: early congenital syphilis, which is diagnosed before the age of two and includes stillbirth, hepatomegaly, rhinitis, or skin tags, and late congenital syphilis, which is diagnosed after the age of two and includes deformities of teeth, bone, and nervous system (9, 28, 29). Congenital syphilis is largely a preventable condition that relies on accurate maternal diagnosis and effective treatment. Universal antenatal screening is recommended at the first antenatal visit, with repeat testing for those at increased risk (15). In the absence of antenatal care, the diagnosis will often go unrecognized. The diagnosis of congenital syphilis can be challenging, with no single diagnostic test available. There are multiple limitations in the current testing modalities, including maternal antibody transfer obscuring the diagnosis. A combination of maternal serology with an absence of appropriate documented treatment, newborn clinical features, and laboratory testing of the placenta and the newborn is required (7, 9). However, given the testing limitations, turnaround time, and the impact of missing a diagnosis, clinical judgement will often dictate treatment regardless of the results from laboratory testing (28, 29).

The commonly used testing modalities include paired maternal and newborn serology, PCR, and placenta histopathology (7). Cord blood should not be used, as it could contain maternal blood, resulting in false positives (9). DFM and immunofluorescence have been largely replaced by the more sensitive method of PCR. All treponemal-based IgG tests are of little value in the newborn due to maternal transfer of IgG (9). Instead, parallel testing of non-treponemal antibody (VDRL or RPR) from sera of both the newborn and the mother is performed to establish the diagnosis as evidenced by a minimum 4-fold or higher titer of the newborn compared to the maternal titer (7). This, however, rarely occurs, with a reported sensitivity of between 4% and 13%. Its absence does not exclude the diagnosis (29). Anti-treponemal IgM does not cross the placenta. If detected in neonatal serum, it is suggestive of congenital infection, but a nonreactive result does not exclude the diagnosis (29). A review on the utility of the EIA IgM in diagnosing congenital syphilis suggested that a reactive result did not influence

diagnosis or treatment, as in most cases, the decision to treat was made before the IgM result was available (29).

Molecular diagnosis has a complementary and expanding role with a range of specimens suitable for testing depending on the clinical signs. Placental tissue, amniotic fluid, neonatal nasal discharge, CSF, and blood may be appropriate sample types. The sensitivity and specificity depend on the targets used. Sensitivities using the Tpp47 target are 75% to 100% for amniotic fluid, 60% to 75% for neonatal CSF, and 67% to 94% for neonatal whole blood or serum compared to that of culture (15). Histologic examination of the placenta shows a traditional triad of enlarged hypercellular villi, proliferative villous vasculature, and villitis. As previously mentioned, immunohistochemistry or silver stains to visualize the spirochetes may also be used (30).

Serologic follow-up at 3 and 6 months is recommended for monitoring response posttreatment as well as in suspected cases with a normal clinical examination and investigation (31). The diagnosis can be excluded at 6 months if the nontreponemal tests become nonreactive in an untreated child. Transmitted maternal serology may remain detected for 15 months in the child. Detection after this period implies congenital syphilis (28).

NEUROSYPHILIS

Central nervous system (CNS) involvement can occur early in syphilis with a predominant meningeal and vascular involvement or may also be a late complication of infection with brain parenchymal and spinal cord involvement (3, 9). Neurosyphilis was commonly described in the preantibiotic era, and currently, CNS infections are most commonly seen in HIV-coinfected patients. In 2017, the incidence of neurosyphilis in the United States was estimated at 9.5 cases per 100,000 persons and was reportedly twice as common in HIV-coinfected patients than in those with syphilis alone (32).

The clinical presentation varies and is determined by the structures involved and duration of infection. The absence of gold-standard tests and the broad clinical features contribute to difficulties in diagnosis. The laboratory diagnosis of neurosyphilis requires serum and CSF examination in the context of a consistent clinical syndrome.

Serological evidence of current or past infection with *T. pallidum* is required to diagnose neurosyphilis (33). As described earlier, treponemal antibody testing is expected to remain positive for prolonged periods following infection, but nontreponemal antibody testing may be negative in late neurosyphilis. If treponemal antibodies are nonreactive, then CSF examination should not be pursued (9, 34). The decision to progress to lumbar puncture in the setting of a reactive treponemal antibody relies on the presence of clinical signs or symptoms. Although up to one-third of patients with neurosyphilis are asymptomatic, there is no published evidence of improved outcomes associated with enhanced treatment in these patients; therefore, the United States Centers for Disease Control and Prevention does not recommend lumbar puncture in the absence of neurological symptoms (34).

The CSF findings of neurosyphilis include pleocytosis, elevated protein, and a reactive CSF-VDRL. A CSF pleocytosis of >5 cell/mm^3 has a sensitivity of 95% but lacks specificity, especially in the setting of HIV coinfection. An elevated CSF protein level of >45 mg/dl is nonspecific and less sensitive than pleocytosis (sensitivity of 90%) (32, 35).

CSF-VDRL is the most widely used nontreponemal test for neurosyphilis because of a reported 100% specificity in the absence of blood contamination (9, 35). A positive CSF-VDRL result is considered diagnostic of neurosyphilis. However, the sensitivity of CSF-VDRL is poor, reportedly between 30% and 70% (32). Therefore, a negative result does not exclude the diagnosis (2, 10). The CSF-RPR test is not recommended due to even lower sensitivity (32, 35).

The FTA-ABS can also be performed on CSF but is performed infrequently and only recommended when CSF-VDRL is nonreactive (2, 32). CSF-FTA-ABS has high sensitivity ($\sim$99%) but low specificity for neurosyphilis due to passive transfer of anti-treponemal

antibodies from blood into the CSF (2, 32, 35). A nonreactive CSF-FTA-ABS result makes the diagnosis of neurosyphilis unlikely. False positives may occur due to blood contamination (32).

T. pallidum PCR has also been assessed for the diagnosis of neurosyphilis. The performance has not been as efficacious as expected. A study using a nested PCR targeting the *tpp47* gene only gave a sensitivity of 42.5% with a specificity of 97% compared with a diagnosis based on clinical assessment and preexisting CSF diagnostic tests (36). Consequently, neurosyphilis is not excluded by negative *T. pallidum* DNA on CSF.

The 2018 CDC case definitions for neurosyphilis provide a helpful summary of the combined utility of each of the above-mentioned tests for neurosyphilis (33).

CONCLUSIONS

Multiple methods are available for the laboratory diagnosis of syphilis, but their utility is restricted by the nature of the disease and the methods' inherent limitations. The laboratory diagnosis of syphilis remains predominantly serological. The most widely used serological assays are treponemal utilized in combination with nontreponemal assays to determine disease activity. It is suggested that two treponemal assays, for example, EIA/CLIA IgG and TPPA, be initially used to confirm the presence of syphilis. All serological assays have variable false-positive and false-negative rates; therefore, a clinical and epidemiologic context needs to be considered in conjunction with the result. Point-of-care tests are available and best used as a screening test in high-prevalence populations with limited formal diagnostic facilities. Molecular detection has a role particularly in placental testing for congenital syphilis. It does not, however, replace serology. The laboratory diagnosis of congenital syphilis and neurosyphilis relies heavily on preexisting serology, clinical features, and treatment history. Invariably, good clinical judgment is paramount, especially when the test result is inconclusive, as frequently seen in complex cases such as neurosyphilis or congenital syphilis. This review promotes the rational use of pathology testing for the many facets of syphilis.

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